

## Four new species of the *Stegana ornatipes* species group (Diptera: Drosophilidae) from Yunnan, China, with DNA barcoding information

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### Abstract

Four new species of *Stegana (Steganina) ornatipes* species group are found from Yunnan, China: *S. (S.) angustifoliacea* sp. nov., *S. (S.) crinata* sp. nov., *S. (S.) nigripes* sp. nov. and *S. (S.) polysphyra* sp. nov. The DNA sequences of the mitochondrial *COI* gene with BOLD Process ID and GenBank accession numbers are provided for the Chinese species.

**Key words:** Barcoding, drosophilid fauna, East Asia, *ornatipes* group, taxonomy

### Introduction

Up to the present, a total of 153 species of the subgenus *Stegana (Steganina)* has been reported from the World (Bächli 2014; Lu *et al.* 2011a; Wang *et al.* 2013; Zhang *et al.* 2014), 76 of them from Chinese part of the Oriental region, which shows that southern China might be an important area in the origin and early radiations of the subgenus *Steganina*. The *Stegana ornatipes* species group is as it were endemic to the Oriental region, and including 11 species described (Cheng *et al.* 2009; Lu *et al.* 2011b), only one species, *Stegana ornatipes* Wheeler & Takada, 1964 is distributed in Micronesia. Recently, based on DNA sequence data of two mitochondrial genes, cytochrome oxidase subunit I (*COI*) and NADH dehydrogenase subunit 2 (*ND2*), phylogenetic relationships among eight species of the *ornatipes* group were analyzed (Lu *et al.* 2011); in addition, Zhang *et al.* (2014) reported 28 DNA barcoding fragments of the mitochondrial *COI* gene for 17 species of the genus *Stegana* from Taiwan, including five samples of two *ornatipes* species: *S. chitouensis* Sidorenko, 1998 and *S. ornatipes* Wheeler & Takada, 1964.

In this paper, four new species of the *ornatipes* group are described from Yunnan, southwestern China. In addition, a total of 12 DNA sequences of the mitochondrial *COI* gene for eight Chinese species of the *ornatipes* group are provided with BOLD Process ID and GenBank accession numbers (Table 1). Up to the present, a total 26 mtDNA *COI* sequences of 14 species of the *ornatipes* group have been reported from China plus in Lu *et al.* (2011) and Zhang *et al.* (2014).

### Material and methods

**Materials and morphological terminology.** All specimens examined were collected by sweeping on tree trunks or tussocks nearby streams in the forest, preserved in 75% ethanol, and then dried and pinned after morphological examination and identification. The specimens are deposited in Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). We followed Zhang & Toda (1992) and Chen & Toda (2001) for the definitions of measurements, indices and abbreviations.

**DNA extraction, sequencing and sequence alignment.** A total of 12 representative individuals belonging eight species were employed for DNA sequencing of the mitochondrial *COI* gene (Table 1). For this purpose, total DNA was extracted from each fly using the TIANGENT™ DNA extraction kit following manufacturer's

instructions. The *COI* fragments were amplified using the cycle protocol as in Zhao *et al.* (2009). The PCR/sequencing primer pair were 5'-CGCCTAAACTTCAGCCATT-3' (Wang *et al.* 2006) and 5' -TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). The PCR products were purified and then directly sequenced on Takara sequencer. The obtained nucleotide sequences were translated into amino acid sequences to ensure their integrity and accuracy, and then aligned with the ClustalW as implemented in MEGA 5.05 (Tamura *et al.* 2011) to rectify the nucleotide level alignment. Our *COI* sequences contained 593 to 710 base pairs in length; the obtained sequences were submitted to the BOLD and the GenBank (Table 1).

**TABLE 1.** Details of the Chinese samples using in the *COI* sequencing and accession numbers.

Species	Localities	BOLD Process ID	GenBank accession numbers
<i>S. albiventralis</i> Cheng <i>et al.</i> , 2009 (♂)	Hesong, Menghai, Yunnan	BDORP001-14	KP179306
<i>S. angustigena</i> Cheng <i>et al.</i> , 2009 (♂)	Wangtianshu, Mengla, Yunnan	BDORP002-14	KP179307
<i>S. angustigena</i> Cheng <i>et al.</i> , 2009 (♂)	Muyiji Park, Ximeng, Yunnan	BDORP003-14	KP179308
<i>S. lingnanensis</i> Cheng <i>et al.</i> , 2009 (♂)	Yixiang, Pu'er, Yunnan	BDORP004-14	KP179309
<i>S. zhaofengi</i> Cheng <i>et al.</i> , 2009 (♂)	Wangtianshu, Mengla, Yunnan	BDORP005-14	KP179310
<i>S. angustifoliacea</i> sp. nov. (♂)	Yixiang, Pu'er, Yunnan	BDORP006-14	KP179311
<i>S. crinata</i> sp. nov. (♂)	Hesong, Menghai, Yunnan	BDORP007-14	KP179312
<i>S. nigripes</i> sp. nov. (♂)	Hesong, Menghai, Yunnan	BDORP008-14	KP179313
<i>S. nigripes</i> sp. nov. (♂)	Baihualing, Baoshan, Yunnan	BDORP009-14	KP179314
<i>S. nigripes</i> sp. nov. (♀)	Hesong, Menghai, Yunnan	BDORP010-14	KP179315
<i>S. polysphyra</i> sp. nov. (♂)	Yixiang, Pu'er, Yunnan	BDORP011-14	KP179316
<i>S. polysphyra</i> sp. nov. (♂)	Hesong, Menghai, Yunnan	BDORP012-14	KP179317

### *Stegana (Steganina) ornatipes* species group

*Stegana (Steganina) ornatipes* species group: Cheng, Gao & Chen 2009: 38.

**Diagnosis.** Surstylus with several thin, long setae and 1 strong prensiseta at anterior apex (Figs 1–4B), rare present on base (Fig. 3B); 10th sternite mostly narrow, nearly arcuate, with 1 pair of projections posterolaterally (Figs 1–4C); gonopods (dorsal arch in Bächli *et al.* 2004) with 1 pair of projections sublaterally (Figs 1–4E); aedeagus mostly ciliate and separated to leafiness from basally (Figs 1–4D).

In the new species described here, only characters that depart from the universal description given by Cheng *et al.* (2009) for this group are provided for brevity.

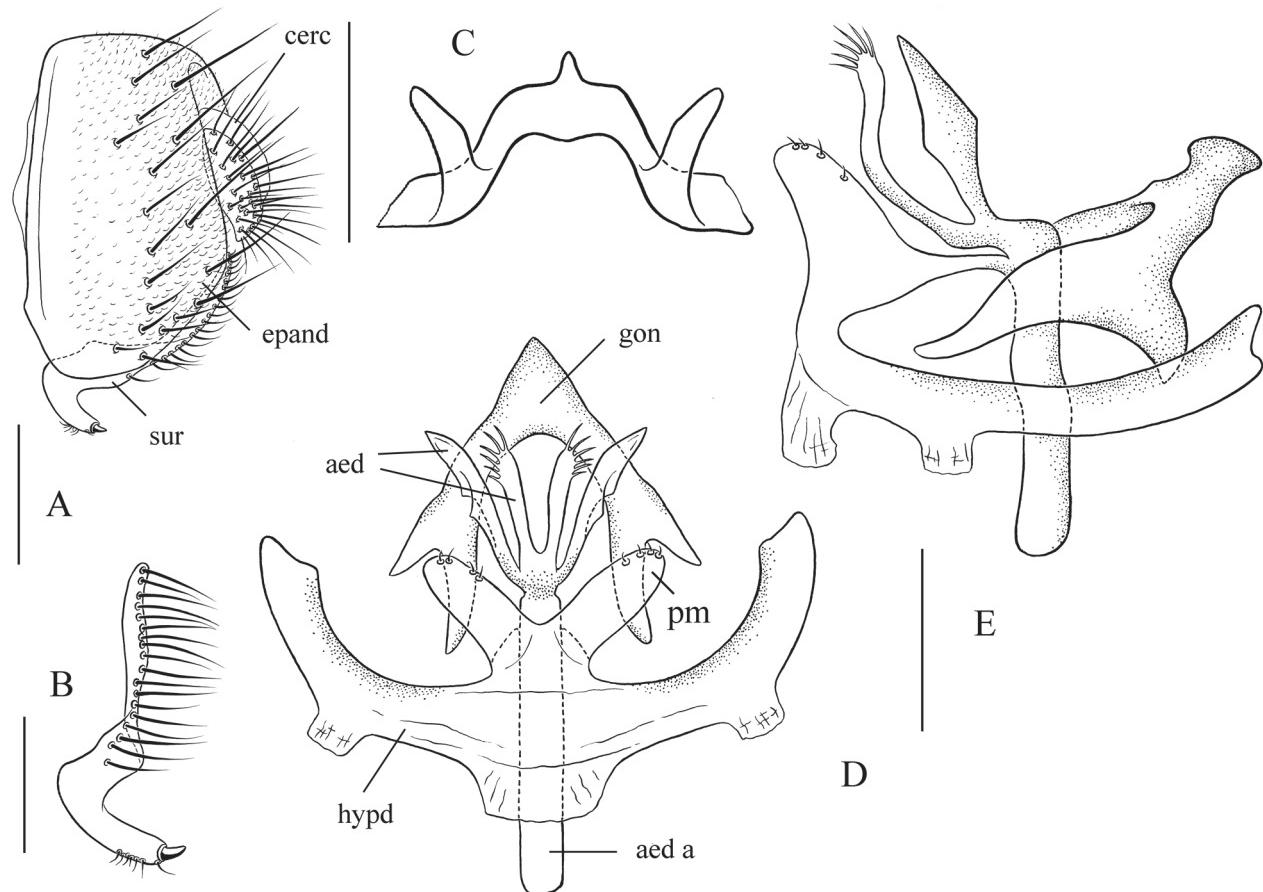
### *Stegana (Steganina) angustifoliacea* sp. nov.

(Fig. 1)

**Diagnosis.** This species resembles *S. vietnamensis* Sidorenko, 1997 in the male terminalia (Fig. 1), but can be distinguished by the colored pattern of katepisternum and shape of gonopods; in *S. vietnamensis*: katepisternum brown on upper part; gonopods round apically (fig. 5 in Sidorenko 1997).

**Description.** Male and female: Pedicel grey-yellow; first flagellomere black on distal 1/2. Palpus yellow, slightly brownish on distal 1/3. Mesonotum and subscutellum entirely brown. Katepisternum entirely white-yellow. Legs yellow, brown to dark brown on basal parts of all femora and distal part of all tibiae. Fore femur with 2 (female)–4 (male) setae on distal half of ventral surface. All abdominal tergites brown medially, yellow laterally; all sternites yellow. Male terminalia: Epandrium with approximately 19 setae near posterior margin on each body-side (Fig. 1A). Cercus lacking pubescence (Fig. 1A). Surstylus slender, anteriorly with finger-like projection (Fig.

1B). Tenth sternite with 1 small projection apico-medially (Fig. 1C). Plate between hypandrium and aedeagus (paraphysis) with 4 sensilla (Fig. 1D, E). Aedeagus composed of 1 bilobed medial process with long pubescence and 1 pair of apically acute, rod-like processes (Fig. 1D, E). Gonopods slightly triangular in ventral view (Fig. 1D, E).



**FIGURE 1.** *Stegana (Steganina) angustifoliacea* sp. nov., male genitalia: A. Epandrium (epand), surstylus (sur) and cercus (cerc) (lateral view); B. surstylus (ventral view); C. 10th sternite (ventral view); D. hypandrium (hypd), paramere (pm), aedeagus (aed) and aedeagal apodeme (aed a) (ventral view); E. ditto (lateral view). Scale lines = 0.1 mm.

**Measurements.** BL = 2.67 mm in holotype (2.77 mm in 1♀ paratype), ThL = 1.20 mm (1.27 mm), WL = 2.33 mm (2.20 mm), WW = 1.07 mm (1.00 mm), arb = 8/5 (7/6), avd = 1.22 (1.09), adf = 1.20 (1.25), flw = 2.20 (2.14), FW/HW = 0.37 (0.38), ch/o = 0.17 (0.16), prorb = 1.05 (1.00), rcorb = 0.58 (0.63), orbito = 1.36 (1.53), vb = 0.35 (0.38), dcl = 0.42 (0.48), presctl = 0.58 (0.56), sctl = 1.12 (1.21), sterno = 0.80 (0.76), dep = 0.23 (0.23), sctlp = 1.22 (1.33), C = 1.95 (1.80), 4c = 1.12 (1.25), 4v = 1.47 (1.44), 5x = 1.29 (1.30), ac = 9.50 (9.80), M = 0.53 (0.56), C3F = 0.63 (0.61).

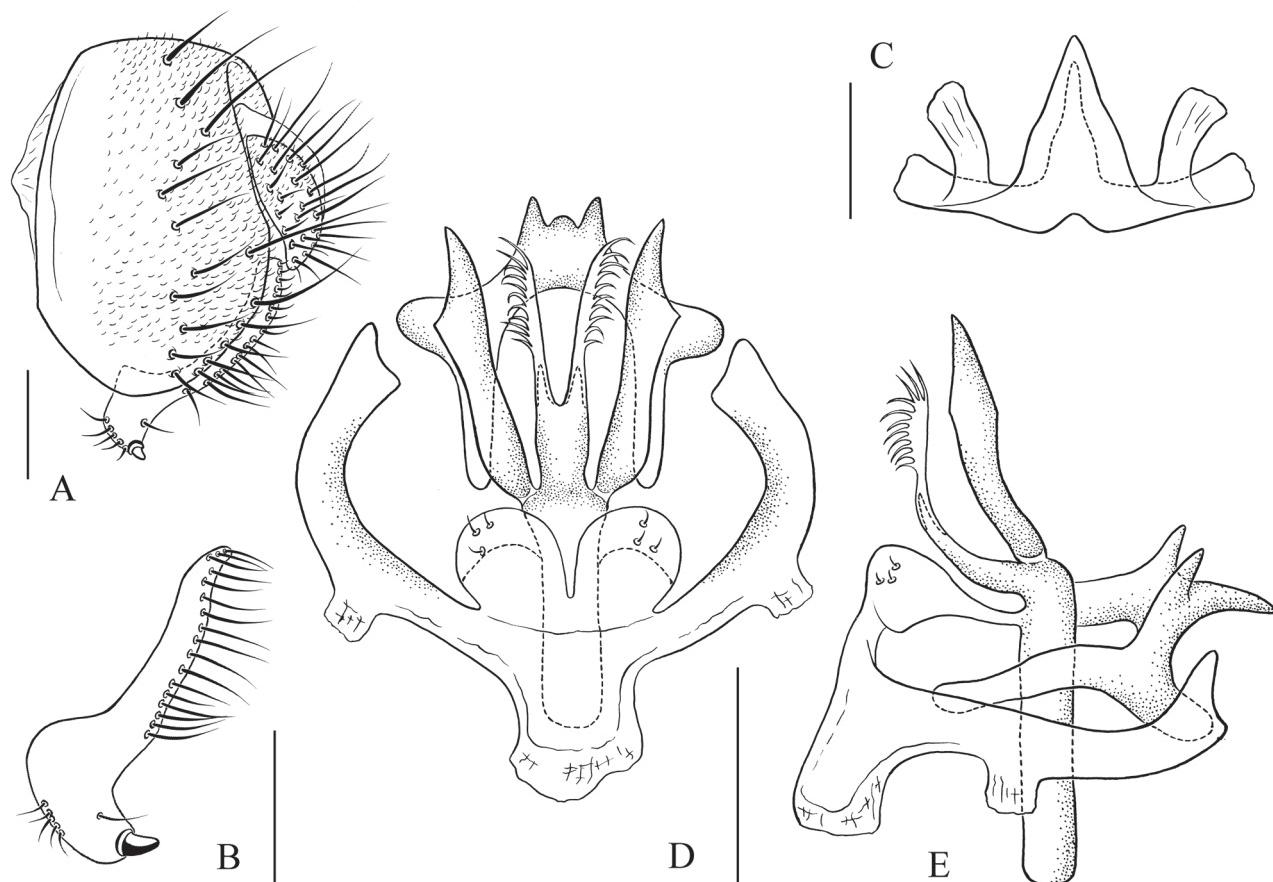
**Specimens examined.** Holotype: ♂ (SCAU, No. 124157), CHINA: Yixiang, Pu'er, Yunnan, 22°42' N, 101°09' E, altitude 1400 m, 13.v.2012, tree trunk, HW Chen. Paratype: 1♀ (SCAU, Nos 124158), same data as holotype.

**Etymology.** A combination of the Latin words “*angustus*” (= narrow) + “*foliaceus*” (= leaf-like), referring to the slender surstylus.

#### *Stegana (Steganina) crinata* sp. nov. (Fig. 2)

**Diagnosis.** This species resembles *S. angustifoliacea* sp. nov. in the male terminalia (Fig. 2), but can be distinguished by the 10th sternite triangular medially (Fig. 2C), the gonopods having 1 pair of acute processes ventroapically (Fig. 2D, E).

**Description.** Male and female: Pedicel grey-yellow; first flagellomere grey-yellow, black on distal 1/3. Palpus yellow basally, slightly brown on distal 1/4. Mesonotum and subscutellum entirely brown. Katepisternum entirely white-yellow. Legs yellow, brown to dark brown on basal parts of all femora and distal part of all tibiae. Fore femur with 2–4 setae on distal half of ventral surface. All abdominal tergites brown medially, yellow laterally; all sternites yellow. Male terminalia: Epandrium with approximately 17 setae near posterior margin on each body-side (Fig. 2A). Cercus with pubescence (Fig. 2A). Surstylus slender, anteriorly with isodiametric projection (Fig. 2B). Plate between hypandrium and aedeagus with 3 sensilla (Fig. 2D, E). Aedeagus composed of 1 bilobed pubescent process and 1 pair of slender, apically acute, rod-like processes (Fig. 2D, E). Gonopods curved dorsad apically (Fig. 2E).



**FIGURE 2.** *Stegana (Steganina) crinata* sp. nov., male genitalia: A. Epandrium, surstylus and cercus; B. surstylus; C. 10th sternite; D. hypandrium, paramere, aedeagus and aedeagal apodeme; E. ditto. Scale lines = 0.1 mm.

**Measurements.** BL = 2.67 mm in holotype (range in 1♂ and 2♀ paratypes: 2.73 mm in ♂, 2.93–3.00 mm in ♀), ThL = 1.33 mm (1.33 mm in ♂, 1.40–1.47 mm in ♀), WL = 2.13 mm (2.20 mm in ♂, 2.13–2.33 mm in ♀), WW = 1.07 mm (1.13 mm in ♂, 1.27 mm in ♀), arb = 6/5 (6/5), avd = 0.90 (0.80–1.00), adf = 1.25 (1.00–1.50), flw = 1.75 (1.63–1.88), FW/HW = 0.35 (0.33–0.34), ch/o = 0.13 (0.13–0.14), prorb = 1.13 (0.91), rcorb = 0.73 (0.68–0.73), orbito = 2.00 (2.00–2.20), vb = 0.38 (0.33–0.40), dcl = 0.33 (0.40), presctl = 0.39 (0.40–0.43), sctl = 1.75 (1.56–1.82), sterno = 0.83 (0.80), dcp = 0.23 (0.20–0.22), sctlp = 1.09 (1.00–1.25), C = 2.05 (1.68–2.00), 4c = 1.06 (1.11–1.32), 4v = 1.39 (1.39–1.74), 5x = 1.14 (1.33–1.57), ac = 9.50 (10.00–12.50), M = 0.44 (0.44–0.63), C3F = 0.58 (0.59–0.71).

**Specimens examined.** Holotype ♂ (SCAU, No. 124150), CHINA: Hesong, Menghai, Yunnan, 21°49' N, 100°06' E, altitude 1700 m, 6, 7.iv.2011, on tussock, JJ Lu. Paratypes: CHINA: 1♂, 2♀ (SCAU, Nos 124151–53), same data as holotype.

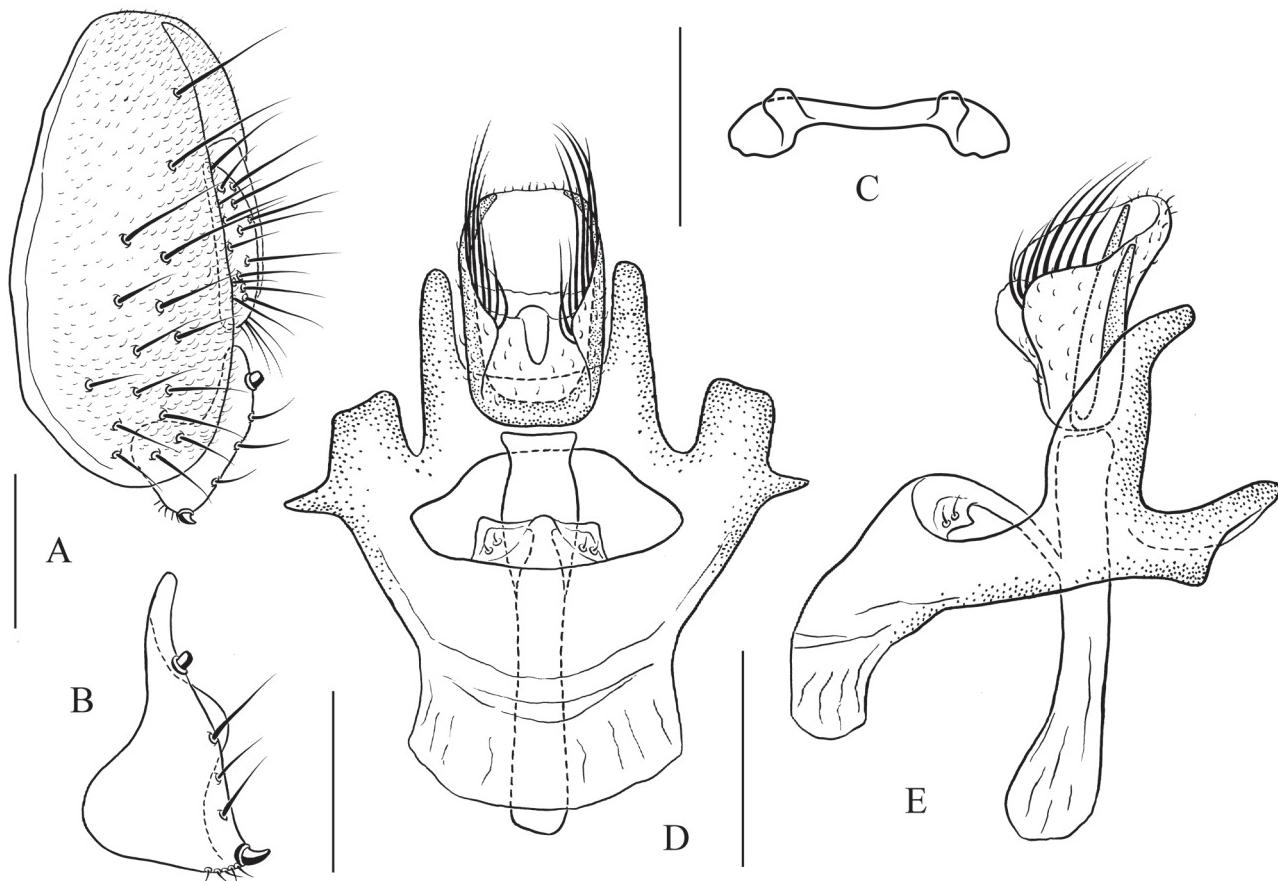
**Etymology.** From the Latin word: *crinatus*, referring to the bristled aedeagus.

*Stegana (Steganina) nigripes* sp. nov.

(Fig. 3)

**Diagnosis.** This species differs from the other species of this group in having the katepisternum dark brown; surstyli with 1 strong prensiseta each apically and subbasally (Fig. 3B); aedeagus ventrolaterally expanded ventrad, with sporadic pubescence and 1 row of long setae apico-laterally (Fig. 3D, E).

**Description.** Male and female: Pedicel and first flagellomere mostly grey-yellow, with black pubescence. Palpus yellow, black on distal 1/3. Legs mostly brown, white on basal parts of fore femur and all tibiae. All abdominal tergites and sternites dark brown. Male terminalia: Epandrium with approximately 16 setae near posterior margin on each body-side (Fig. 3A). Cercus lacking pubescence (Fig. 3A). Plate between hypandrium and aedeagus with 2 sensilla (Fig. 3D, E). Gonopods basally coalescent with the lateral arms of hypandrium, broadened dorso-medially, with 1 projections sublaterally each side (Fig. 3D, E).



**FIGURE 3.** *Stegana (Steganina) nigripes* sp. nov., male genitalia: A. Epandrium, surstylus and cercus; B. surstylus; C. 10th sternite; D. hypandrium, paramere, aedeagus and aedeagal apodeme; E. ditto. Scale lines = 0.1 mm.

**Measurements.** BL = 2.33 mm in holotype (range in 1♂ and 1♀ paratypes: 3.01 mm in ♂, 3.05 mm in ♀), ThL = 0.87 mm (1.39 mm in ♂, 1.40 mm in ♀), WL = 1.67 mm (2.13 mm in ♂, 2.53 mm in ♀), WW = 0.73 mm (1.04 mm in ♂, 1.23 mm in ♀), arb = 11/5 (8–12/5–6), avd = 1.00 (0.82–0.95), adf = 1.80 (1.11–1.14), flw = 2.20 (1.83–2.08), FW/HW = 0.38 (0.37–0.39), ch/o = 0.17 (0.14–0.16), prorb = 1.07 (0.78–0.99), rcorb = 0.67 (0.71), orbito = 1.36 (1.46–1.50), vb = 0.50 (0.40–0.42), dcl = 0.55 (0.50–0.67), presctl = 0.59 (0.44–0.67), sctl = 1.32 (1.05–1.61), sterno = 0.71 (1.00), dcp = 0.24 (0.21), sctlp = 1.38 (1.43–1.61), C = 1.81 (1.86–2.29), 4c = 1.14 (0.87–1.05), 4v = 1.43 (1.62–1.98), 5x = 1.20 (1.02–1.12), ac = 8.00 (6.25–7.08), M = 0.43 (0.38–0.44), C3F = 0.64 (0.48–0.55).

**Specimens examined.** Holotype ♂ (SCAU, No. 124154), CHINA: Hesong, Menghai, Yunnan, 21°49' N, 100°06' E, altitude 1800 m, 7.v.2012, on tussock, HW Chen. Paratypes: CHINA: 1♀ (SCAU, No. 124155),

21.iii.2011, JM Lu, same data as holotype; 1♂ (SCAU, No. 124156), Baihualing, Baoshan, Yunnan, 25°18'33 N, 98°48'03 E, altitude 1400 m, 20.vi.2013, on tussock, QS Gao.

**Etymology.** A combination of the Latin words: *niger* and *pes*, referring to the black legs.

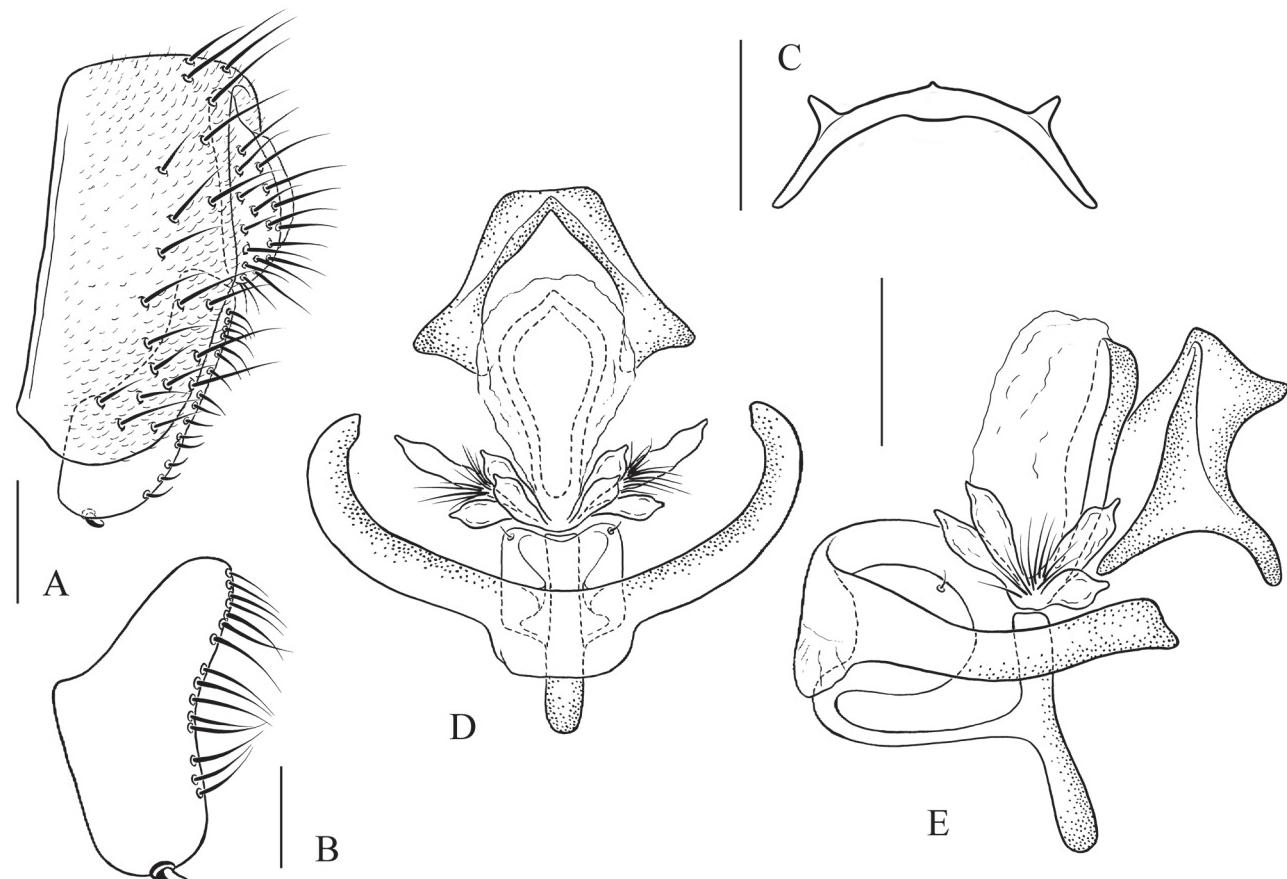
***Stegana (Steganina) polysphyra* sp. nov.**

(Fig. 4)

**Diagnosis.** This species resembles *S. chitouensis* Sidorenko, 1998 in the male terminalia, but can be distinguished by the aedeagus composed of 1 circinate, dorsal process and 4 pairs of rod-like, ventral processes with several setae basally (Fig. 4D, E); in *S. chitouensis*: aedeagus trilobed into dorsal rod and a pair of strongly sclerotized, ventral rods with dense pubescence and 4 pairs finger-like processes (fig. 4B, C in Zhang *et al.* 2014).

**Description.** Male: Pedicel and first flagellomere nearly black. Palpus yellow, dark brown on distal 1/4. Mesonotum brown, with 2 indistinct, thin, black longitudinal stripes sublaterally. Scutellum brown to dark brown. Katepisternum dark brown on upper part, yellow on lower part. Legs yellow, black on knee of forelegs, mid and hind femora and all 1st tarsomeres. Male terminalia: Epandrium with approximately 21 setae near posterior margin on each body-side (Fig. 4A). Cercus lacking pubescence (Fig. 4A). Tenth sternite with 1 small projection apico-medially (Fig. 2C). Plate between hypandrium and aedeagus with 1 sensillum (Fig. 4D, E). Gonopods broadened and protruded dorsad apico-medially (Fig. 4E).

**Measurements.** BL = 3.00 mm in holotype (2.95 mm in 1♂ paratype), ThL = 1.27 mm (1.47 mm), WL = 2.20 mm (2.33 mm), WW = 1.07 mm (1.01 mm), arb = 6/5 (8/5), avd = 0.90 (0.75), adf = 1.25 (1.50), flw = 1.50 (1.50), FW/HW = 0.32 (0.37), ch/o = 0.13 (0.10), prorb = 1.00 (0.91), rcorb = 0.60 (0.85), orbito = 2.00 (1.80), vb = 0.47 (0.45), dcl = 0.47 (0.30), presctl = 0.59 (0.60), sctl = 1.54 (1.26), sterno = 0.84 (0.90), dcp = 0.23 (0.26), sctlp = 1.36 (1.33), C = 2.00 (1.70), 4c = 1.25 (1.20), 4v = 1.33 (1.33), 5x = 1.43 (1.43), ac = 6.67 (8.00), M = 0.67 (0.60), C3F = 0.63 (0.67).



**FIGURE 4.** *Stegana (Steganina) polysphyra* sp. nov., male genitalia: A. Epandrium, surstyli and cercus; B. surstyli; C. 10th sternite; D. hypandrium, paramere, aedeagus and aedeagal apodeme; E. ditto. Scale lines = 0.1 mm.

**Specimens examined.** Holotype ♂ (SCAU, No. 124148), CHINA: Yixiang, Pu'er, Yunnan, altitude 1400 m, 2.x.2011, on tree trunk, HW Chen. Paratype: CHINA: 1♂ (SCAU, No. 124149), Hesong, Menghai, Yunnan, 21°49' N, 100°06' E, altitude 1800 m, 7.v.2012, on tree trunk, HW Chen.

**Etymology.** A combination of the Greek words: *poly* + *sphyra*, referring to the shape paramere.

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